Commentary

Reactivity Parameters in Structure–Activity Relationship-based Risk Assessment of Chemicals

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New approaches to the risk assessment process are needed that might be more definitive and satisfying to the scientific community, interest groups, and the public at large. This commentary examines an alternative approach that is based on understanding the relationships of chemical structure and reactivity properties to the toxicokinetic behavior of chemicals in biological systems. This approach is based on the likelihood that there is a limited number of triggering (reactivity) mechanisms by which chemicals can express their toxicity at the molecular level. The fundamental importance of electrophilic character of chemicals as a determinant of their critical molecular reactivities and interactions with biological material in the expression of toxicity is supported. Such an approach also takes advantage of the maturing field of theoretical/computational chemistry in understanding important molecular recognition and reactivity processes (both qualitatively and quantitatively) for chemicals that can underlie their biological/toxicological activity. A process that permits assessment of reaction equivalents delivered to biological systems may hold promise for grouping chemicals by common triggering mechanisms with clearly delineated toxicological endpoints. *Key words*: chemical reactivity, molecular mechanisms, protein binding, risk assessment, structure–activity relationships, toxicity. *Environ Health Perspect* 104:810–816 (1996)

The analytical tools of risk assessment as applied to chemicals (*1*) have assumed a critical role in decision making in the United States. In light of the growing importance of risk assessment, it is disconcerting that America's current risk assessment processes do not command a high degree of respect within the scientific community, interest groups, and the public at large. There is clearly a need to pursue other approaches to the risk assessment process that might be more definitive and satisfying. This commentary examines another approach that is deep seated in understanding the relationships of chemical structure and reactivity properties to the toxicokinetic behavior of chemicals in biological systems. Such an approach also takes advantage of the coming of age of theoretical chemistry (*2*) in understanding important molecular recognition and reactivity processes for chemicals that can underlie their biological activity. The specific role of computational chemistry in support of hazard identification and mechanism-based structure–activity relationships (SARs) has been recently reviewed (*3*).

This approach is based on the likelihood that there is a limited number of triggering (reactivity) mechanisms by which chemicals can express their toxicity at the molecular level (Fig. 1). While many triggering or bioactivating events may be multifactorial in nature and present higher order complexity as a whole, those ultimately resulting in a given mechanistically defined toxic endpoint are likely to be limited in number. This is expected to be especially important for specific as opposed to nonspecific types of toxicity. In this context, specific toxicity is defined as toxicity that is more a property of substructural features of molecules, such as unique reactivity, than bulk or overall structural properties. For example, it is known that the two polychlorinated biphenyl congeners 3,3′,4,4′-trichlorobiphenyl (3,3′,4,4′-TCB) and 2,2′4,4′,5,5′-hexachlorobiphenyl (2,2′4,4′,5,5′-HCB) have remarkably different acute toxicities in the guinea pig, but their elimination kinetics in this animal species are quite similar (*4*). We now know that this difference in toxicity is largely due to the dioxinlike character of the 3,3′,4,4′-TCB and its ability to attain a coplanar state, facilitating binding to the dioxin receptor family of binding proteins (*5*).

Nonspecific toxicity of the narcotic type, on the other hand, is defined as the converse situation in which toxicity is more often associated with bulk structural properties of chemicals such as lipophilicity or amphiphilicity. An example of this is anesthetic chemicals; amphipathic targets in proteins of the central nervous system can apparently accept molecules with a wide variety of shapes and chemical groupings but with some size limitations (*6*). Noncovalent reactivity properties, as defined later, are used as predictors in some models for narcosis EC_{50} values (concentration required to produce a response in 50% of a population of animals), either directly or indirectly, through the prediction of partitioning behavior estimated by logP.

Chemical Reactivity Classifications

To understand the relationships of chemical structure or substructural features to toxicokinetic properties, it is important to examine some important chemical reactivities that are encoded within the structures of chemicals. Covalent reactivity is associated with forming covalent bonds with other molecules, either directly or through activation to reactive intermediates, which may occur during metabolism (*7*). These systems are often electrophilic in nature and react with nucleophilic sites in target molecules to form conjugates or adducts, such as protein or DNA adducts, formed by genotoxic chemicals. Covalent-type interactions can also occur with some naturally occurring inorganic chemicals such as the metalloid arsenic (*8*).

While electrophilicity is obviously an important consideration in understanding how chemicals cause toxicity, some toxic effects, e.g., carcinogenicity, probably involve multiple mechanisms that include noncovalent molecular reactivities such as their ability to recognize and bind hormone-specific binding sites in tissues and cells. Noncovalent reactivity is associated with nonbonding interactions such as Van der Waals, polarizability, hydrogen bonding forces, etc. This is the type of reactivity that occurs during many enzyme–substrate and ligand–receptor interactions such as the complexation of dioxin with the Ah recep-

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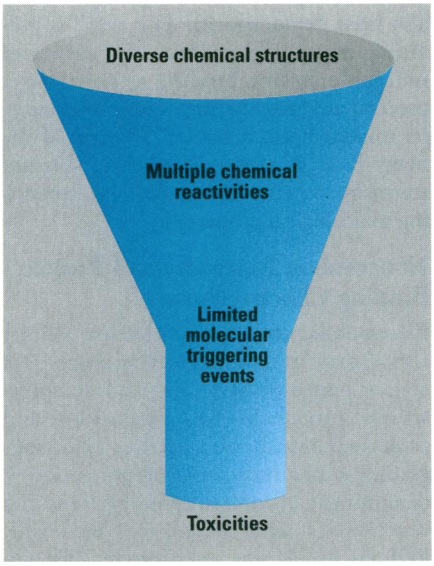

Figure 1. Mechanistically defined toxicities linked with specific molecular triggering events through reactivity considerations.

tor (*9*). Although single point (atom-centered) interactions of this type are generally of lower binding energy than found in covalent bonding, many small interactions that can occur in a ligand receptor complex can produce a tightly bound system with significant stability. Binding of this type can be highly structurally specific, such as in the lock and key type binding often referred to in enzyme–substrate interactions, or can be associated with lower degrees of structural specificity, as in a charge transfer or stacking type interaction.

Ionic reactivity refers to bonding most often associated with cation and anion interactions, redox, and ligand exchange chemistry of inorganic compounds (*10*). This can be especially important in understanding metal cationic toxicokinetics, but it is also important in understanding anionic interactions such as those of the sulfates and nitrates. There are a number of parallels between the biochemical toxicology of organic and inorganic chemicals based on key chemical properties or reactivities that may be common to both. These include metals functioning as alkylating agents; antimetabolites in isomorphous exchange reactions; initiators, catalysts, or depletors in key biochemical processes; and as complexing agents and modulators of redox processes associated with absorption, storage, metabolism, and excretion of metal compounds [for a general review see Hanzlik (*11*)]. However, in attempting to draw parallels between the electrophilic reactivities of metals and organic compounds in toxicity, we learned that much less is known about the normal physiological functioning of metals, as compared to organic compounds, in biological systems. In addition, the range of chemical properties and reactivities offered by metal compounds of various types is considerably greater than that for simple organic compounds. For these reasons, more detailed discussion is limited to the metalloid arsenic, which has electrophilic reactivities expressed through a combination of its redox cycling and covalentlike reactivities.

These three chemical reactivity classifications represent three points along a continuum of bonding interactions in chemical systems. In addition, there are variations of each of these that can complicate any simple understanding of the relationships of chemical structure to chemical and biological reactivity. However, some useful concepts and approaches have been suggested that can help guide our thinking about such problems. An important objective of such analysis would be to attempt to relate certain chemical reactivity subclassifications to specific types or forms of toxicokinetic behavior in biological systems. In this way it may be possible to group some otherwise structurally diverse chemicals into a toxic equivalency-type framework to aid the risk assessment process (*12*).

Approaches to Assessing and Quantitating Electrophilic Character and Covalent Reactivity of Chemicals

The evaluation of electrophilic character has been approached through the principles of acid–base theory where electrophiles (acids) and nucleophiles (bases) can be classified as hard or soft, based on intrinsic characteristics of the center under consideration (*13*). In this scheme, hard species typically have a small atomic radii and a high effective nuclear charge and are only slightly polarizable (e.g., the aluminum cation); soft species tend to be large and highly polarizable (e.g., arsenic). The simple rule of thumb is that hard electron-deficient centers prefer to bind with hard electron-rich centers (such as aluminum and fluoride) and, likewise, soft electron-deficient species prefer soft electron-rich species (such as arsenic and sulfur). This concept can be a powerful approach to correlating chemical facts in the absence of detailed and direct knowledge of the process under study.

The concept can also be applied to evaluate carbon acids (*13*) that may be formed as reactive intermediates during metabolism. For example, in the metabolism of trihalomethanes (THMs) such as chloroform, which occur as by-products of water chlorination, several competing metabolic pathways are possible (*14*) that can yield electrophilic intermediates with varying reactivities and potential for triggering toxicity (Fig. 2). This can include oxidative (phosgene-like and cationic intermediates) and one and two electron reductive (free radical and carbene intermediates) processes, and in some cases the THMs can be sufficiently electrophilic to undergo direct nucleophilic attack by nucleophiles like sulfur [as in glutathione (GSH)]. It is not clear if all THMs would undergo metabolism via all of these different pathways, but there are both theoretical and experimental approaches to assessing the relative reactivities of THMs of interest. For example, theoretical calculations have been applied to estimate the electron affinities of a series of halomethanes, as an indicator of the potential to undergo reductive metabolism (*15*). *In vitro* metabolism studies under aerobic and anaerobic conditions have also been carried out to estimate the flux through oxidative and reductive pathways, as well as the associated covalent binding of intermediates to biological materials (*16*).

In general, the increased reactivity and binding of the brominated THMs predicted by theory have been confirmed experimentally (*16*). In addition, gas uptake chamber experiments have confirmed increased metabolic activity of brominated THMs and suggested increased involvement of first order or pseudo first order processes (*17*). Although it is not yet clear in all cases which pathways and electrophilic intermediates are associated with which toxicities, it should be possible to make estimates of the relative fluxes and importance of each pathway in leading to a combined reactivity potential for THMs via a given electrophilic species.

One approach to this problem has been to isolate and separately study each pathway to the extent possible. For example, THM mutagenicity mediated by reaction with GSH has been assessed using a strain of *S. typhimurium* TA1535, which was transfected with rat glutathione *S*-transferase (GST) (*18*). In this study, bromoform was most active, followed by bromodichloromethane and chloroform (relatively inactive). These results are consistent with estimated electrophilicities based on calculations of vertical electron affinities (*15*) and indicate that bromination confers the capability for GST-mediated transformation to mutagenic intermediates. The *in vivo* genotoxic potential of the BrTHMs may have been underestimated (*18*). Theoretical predictions of such reactivities can be complicated by other factors such as the potential steric hindering effects of hav-

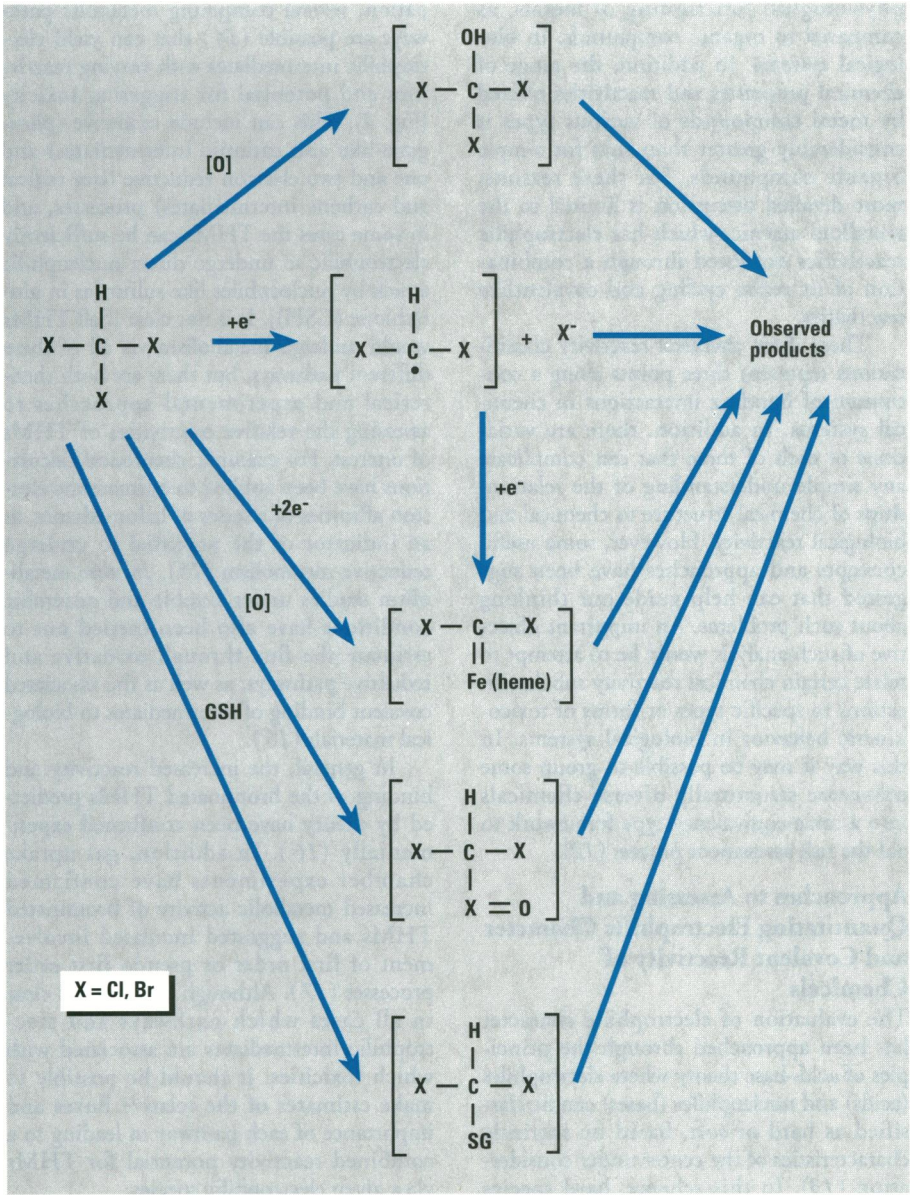

Figure 2. Possible chemical mechanisms for trihalomethane metabolism involving electrophilic intermediates with varying reactivities and potentials for triggering toxicity.

ing multiple large bromine atoms on the same carbon atom. Nevertheless, studies such as this are useful in linking a mechanistically defined toxic endpoint with a specific molecular triggering event.

The covalentlike reactivity of inorganic arsenic is also predicted on the basis of hard-soft acid–base theory, and it is anticipated that sulfur will play an important role in its ligand exchange chemistry. Furthermore, the redox chemistry of inorganic arsenic suggests that pentavalent arsenic (As^5) would be a strong oxidant and would prefer to oxidize its potential ligands rather than complex them where it is possible (*8*). Once reduced, trivalent arsenic (As^3) can complex with soft bases such as sulfur through ligand exchange chemistry. In addition, trivalent arsenic can donate electrons to electrophilic methylating species. It is anticipated that it would not react with hard bases as found in DNA. Some similarities in the oxyanion chemistry of arsenate and phosphate can also be predicted. These reactivity predictions have been confirmed through studies (Fig. 3) that have shown that As^5 reacts with GSH first by oxidizing it and then by forming trivalent arsenic complexes (*19*). In addition, trivalent arsenic dithiol complexes were shown to be stable end products of such chemistry. Metabolism of arsenic through methylation has been well documented (*8*). Involvement of arsenate as an oxyanion replacement for phosphate has also been demonstrated. This work is providing new insights about the nature of inorganic arsenic binding sites in target proteins that may be involved in mediating its toxicokinetic behavior. In view of the many faces of arsenic chemistry, it is not surprising to also find varied and interesting toxicological properties.

Noncovalent Reactivity and Protein Binding Characteristics

Electrophilic character or electron affinity also appears to be an important property of certain noncovalent receptor/nonreceptor-specific protein binding interactions and associated metabolic activities (*20*), suggesting some fundamental importance as a determinant of molecular interactions and reactivities with biological material. Perhaps this is not so surprising because the cross-section of biomolecules appears to be more electron donating (nucleophilic) than electron accepting (electrophilic) in nature (*21*). Similar considerations can be applied to the study of inorganic toxicants (*10*).

Specific binding interactions between chemicals and proteins can involve a variety of proteins, including both receptor and nonreceptor proteins. In both cases the binding can be stereospecific and low capacity and high affinity in nature. It is anticipated that receptor protein interactions would lead to a biological response which can often be agonistic in nature, implying that the chemical is able to mimic the full range of reactivity properties contained in the natural ligand. Nonreceptor protein interactions that are not normally directly linked to specific biological responses can often lead to inhibitory effects, such as when competitive binding occurs to transport proteins or metabolic enzymes, implying a lower degree of mimicry to simply block access by the natural ligand. Specific interactions of this type can play an important role in mediating or triggering toxic events in biological systems such as those involved in endocrine disruption (*9*). Supramolecular chemistry associated with self-assembling structures is based, to a large extent, on specific noncovalent interactions, finding applications ranging from materials science to medicine such as approaches to a general solution to DNA recognition (*22*).

Specific Protein Binding as Possible Triggering Events

Perhaps the best known and documented example of receptor-mediated toxicity is the toxicity of dioxin and related halogenated aromatic compounds associated

with binding to the dioxin or Ah receptor (*23*). In spite of the extensive study of this receptor, the endogenous ligand for the receptor is not known, although it is considered extremely likely that one exists (*24*). A nonreceptor (prealbumin) binding model has also been developed and studied (*9*) for the same classes of halogenated aromatic hydrocarbons and hydroxylated derivatives.

Quantitative Estimates of the Relevant Noncovalent Reactivities Underlying Specific Protein Binding and Toxicity

Potencies derived from a number of *in vitro* and *in vivo* binding and biological effects studies with structurally related classes of halogenated aromatic hydrocarbons have shown an excellent correspondence in quantitative structure–activity relationships, which support the role of the dioxin receptor in mediating such effects (*25*). In turn, there have been several theoretical models developed in an attempt to explain the structure–activity relationships and the importance of certain structural features of the chemicals (*26*). While many of these models have unique features and advantages, the model (and molecular reactivity) that seems to be most consistent with the range of molecular sizes, shapes, and aromatic nature of all the ligands that are now known to bind this receptor is the model based on stacking and separation parameters (*27*) (Fig. 4). It is clear that although binding to this receptor is a necessary event it is not sufficient for the expression of toxicity (*25*). One relatively simple measure of the stacking capacity of such chemicals is their binding to carbon. This feature alone has been shown to be useful for the analytical separation of PCBs on the basis of the degree of *ortho*-substitution, which determines how well they stack (bind) to a carbon column (*28*). In general, the less *ortho*-substituted (more dioxinlike toxic) PCBs are retained the most. Similar mechanisms operating on stationary phases bonded to silica gel have been used for high pressure liquid chromatographic separation of positional isomers of the chlorinated dioxins (*29*).

Since the highly toxic halogenated aromatic hydrocarbons are substituted in lateral positions (opposite ends of the more or less rectangular shape), it appears that another important reactivity in mediating their toxicity is one which is dependent on laterality of halogen substitution. This is an important, if not the most important, binding feature represented in the prealbumin interaction model (Fig. 5) previously mentioned (*9*). This can be viewed as a molecular cleft-type binding interaction between the highly polarizable lateral halogen atoms and the hydrophobic interior of the cleft provided by amino acid side chains that converge on the halogen substituents. In terms of attractive forces, the atoms in the amino acid side chains cause distortion of the large electron cloud surrounding the lateral chlorines (emphasized in Fig. 5), consequently resulting in an attraction between the electrons in one atom with the positive nucleus of another. For example, the unchlorinated biphenyl molecule itself, which has relatively little

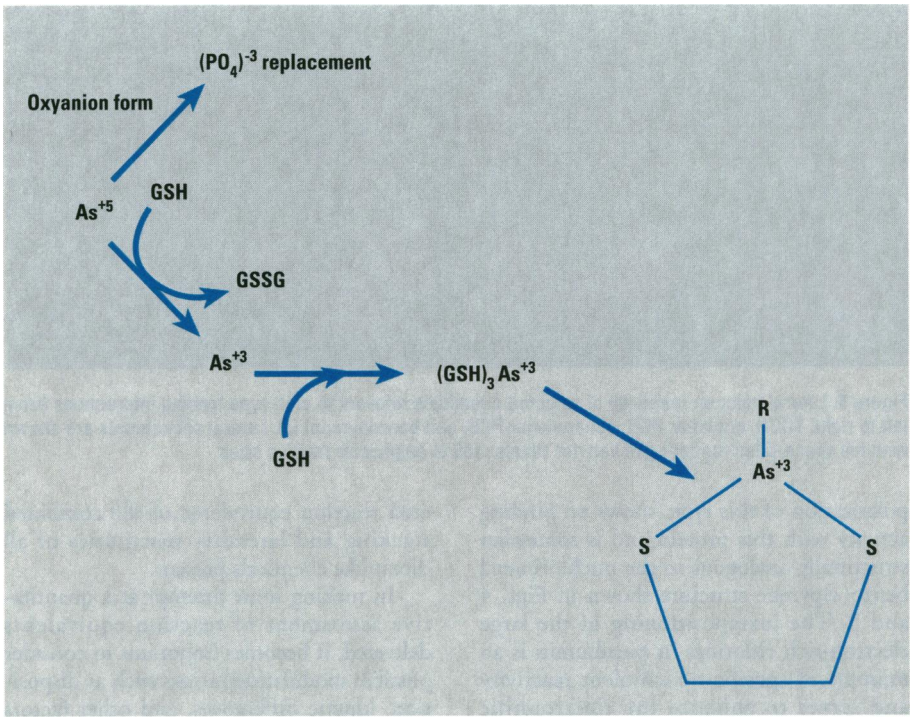

Figure 3. Reductive metabolism of arsenic. Arsenic undergoes reduction from +5 to +3 state. Glutathione (GSH) catalyzes this reduction forming a $(GSH)_3As^3$ complex. $(GSH)_3As^3$ donates As to dithiols; arsenate can also replace phosphate in other pathways.

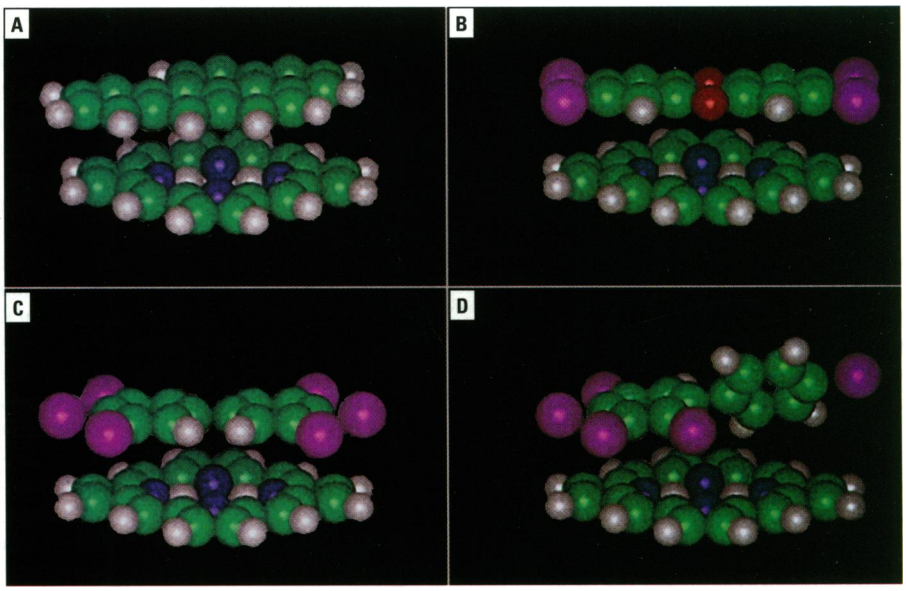

Figure 4. Stacking reactivity of selected chemicals relevant to Ah receptor binding. (A) Benzopyrene; (B) TCDD; (C) coplanar PCB; (D) noncoplanar PCB. The lower porphine ring system is the reference plane; note the separation distance and planar geometrical extent of the stacking interaction involving planar faces.

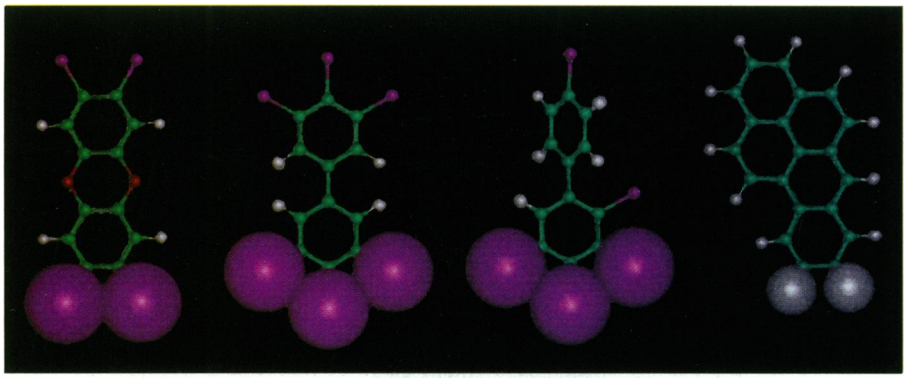

Figure 5. Lateral halogen reactivity of selected chemicals relevant to cleft-type binding interactions (from left to right, TCDD, coplanar PCB, noncoplanar PCB, and benzopyrene) (*9*). Lateral substituents are shown with full space-filled rendering of van der Waals radii to emphasize relative sizes.

polarization of this type, shows no binding activity with this protein and is somewhat structurally analogous to the unchlorinated benzo(*a*)pyrene structure shown in Figs. 4 and 5. The juxtapositioning of the large electron-rich chlorines in prealbumin is an example of specific noncovalent reactivity and serves to enhance the electrophilic reactivity of the positively charged nucleus of the converging atoms in the amino acid side chains. As was the case for the dioxin receptor model, there are both theoretical and experimental counterparts to this model that have predictive value for the laterality reactivity.

The combination of these two reactivity models for the halogenated aromatic hydrocarbons is highly predictive of the potential for dioxinlike toxicity, whereas the use of one or the other reactivity model alone may not necessarily be predictive. For example, of the four aromatic structures illustrated in Figs. 4 and 5, only 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and the coplanar PCB have both strong stacking and laterality reactivity potential.

Modulating Factors in Assessing Reaction Equivalents

An important consideration in risk assessment today is understanding the relationship of exposure to a chemical and the actual dose to target tissue (*30*). This concept is extended here to include assessing the reaction equivalents delivered to target sites in tissues and cells. In the context of exposure to volatile organic compounds (VOCs) such as THMs, this could involve, for example, determining the total reaction equivalents delivered via the phosgenelike (oxidative) metabolic pathway (*31*). For the case of exposure to halogenated aromatic hydrocarbons, this could involve determining the dioxinlike equivalents delivered to target sites (*32*). This in turn could lead to determining the total delivered reaction equivalents of the combined stacking and laterality reactivities of all dioxinlike chemicals present.

In making some attempt at a quantitative assessment of reaction equivalents delivered, it becomes important to consider possible modulating factors such as disposition, kinetic differences, and other factors such as steric accessibility to the target site.

Such factors can not only determine how much of the reactive species is available to target sites but also how well it can bind or interact with the site in effecting a response. Consider the case (Fig. 6) of potential exposure to dioxin or the dioxinlike PCB 3,3′,4,4′-TCB, which is a close isostere of dioxin. In some animal species such as the guinea pig, there do not appear to be major differences in the pharmacokinetic behavior of these two chemicals. But in other species such as the rat, there is potential for significant metabolism of the PCB to a hydroxylated metabolite, which can be eliminated from the body much more readily (*33*). Thus, the availability of the parent PCB to express its reactivity at target sites in the rat is reduced. Estimates of the flux through this metabolic pathway can be measured or estimated from information about the chemical nature of metabolic reactions.

Another important consideration with these two chemicals, even in the absence of differences in metabolism, is their equivalence in terms of the reactivity classifications of importance to the expression of dioxinlike reactivity. Again we can think of this as mainly the combined potential of both the stacking and laterality type binding reactivities. Since all PCBs are basically noncoplanar in nature, it is necessary for the 3,3′,4,4′-TCB to achieve a coplanar state in order to be isosteric with the TCDD structure and facilitate a putative stacking interaction with the receptor. The energy cost to achieve the coplanar state has been estimated to equate to less than 0.5% population of coplanar conformers (*5*). This population, which reflects the stacking reactivity potential for the PCB, can be further reduced by one-half because, of the two possible coplanar conformations, only one is isosteric (similar placement of lateral chlorines) with TCDD. This in effect cuts in half the laterality reactivity potential of the PCB. Consideration of these two factors alone as modulators of the important reactivities goes a long way toward explaining the differences (300–500 fold) in acute toxic potency for this PCB and TCDD in the guinea pig, an animal species in which pharmacokinetic differences does not appear to play a major role (*4*).

Reactivity Parameters in Quantitative Structure–Activity Relationship-based Risk Assessment

The application of reactivity parameters in quantitative structure–activity relationship (QSAR)-based risk assessment is dependent on first classifying important chemical reactivities in a toxicological framework (*34*). This in turn would depend on identifying and quantifying exposures to important reactivity classifications. Reactivity profiles will probably vary both qualitatively and quantitatively with the various environmental media and routes of exposure such as drinking water. Finally, this would require new analytical approaches designed to detect and quantify specific reactivities without necessarily providing chemical specific information. It is hoped that such approaches could also be designed to provide information about reactivities of chemicals derived from metabolic and environmental transformation processes. Some reactivities will undoubtedly be found that are not clearly linked to toxicological endpoints.

There are several advantages to such an approach, even though this would be a significant new direction in the current method of risk assessment of exposure to environmental chemicals (*1*). First, it is anticipated that there would be a more limited (less than the universe of chemicals to which we are exposed) number of important chemical reactivities or molecular triggering mechanisms for toxicity with which to deal, permitting a considerable amount of grouping of chemicals through the use of toxic equivalency factors. Second, this approach should also serve to increase the sensitivity for detecting important chemicals in the environment by providing a combined and amplified reactivity signal to quantitate. Third, development of *in vitro* correlates of reactivities with toxic endpoints could permit rapid screening of envi-

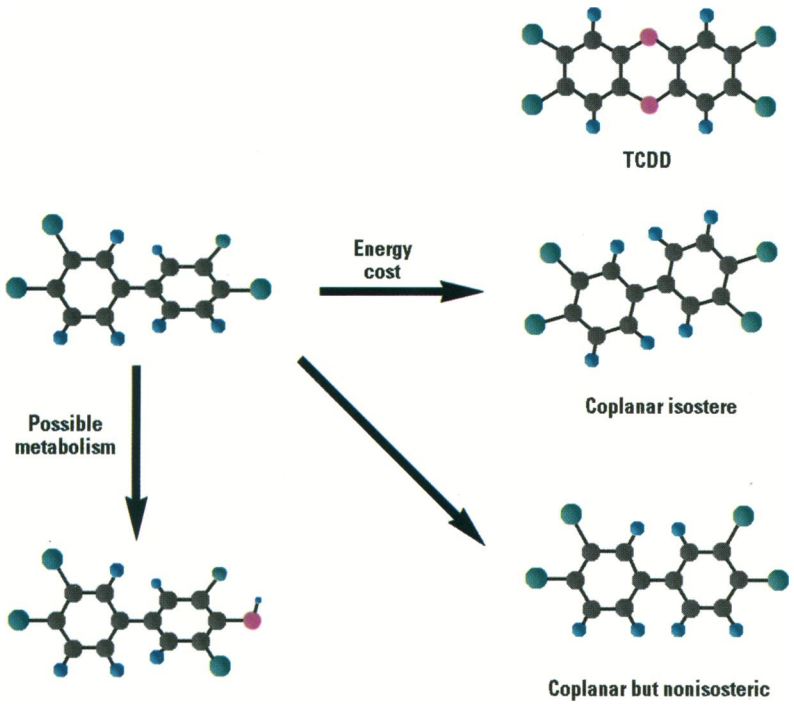

Figure 6. Some factors that can modulate delivery of reaction equivalents in biological systems. TCDD compared with noncoplanar and coplanar (isosteric and nonisosteric) conformers of PCB and the potential for PCB metabolism.

ronmental media for toxic potential by important triggering mechanisms. The use of the Ames test for screening for genotoxicity of mixtures (*35*) is an example of this; however, in the context of this proposal, it can be viewed as a measure of electrophilic reactivity toward hard bases in DNA. This information can be used immediately to initiate possible engineering strategies to remove or reduce undesirable reactivities. A reactivity-based approach could also be used to guide more detailed biological/chemical/mechanistic studies to determine the specific chemicals underlying the reactivities and the development of pharmacokinetic (PB-PK) and dose–response (BB-DR) models to further reduce the uncertainties associated with the risk assessment process. In this way, the reactivity profiles could be used to guide and direct the appropriate research directions and areas of emphasis needed to address potentially important health problems (both ecological and human health) from exposures to environmental chemicals.

Such an approach is obviously limited by current understanding of relationships between chemical reactivity and toxicity. Clearly, more research is needed to discover new chemical reactivity–toxicity correlates. As an example of such a correlate, Bakale and McCreary (*36*) have experimentally measured the electron attachment rate constant (k_e) as an indicator of electrophilic reactivity and proposed this property as a general physico-chemical screening indicator of carcinogenic activity. Benigni et al. (*37*) developed a theoretical method that permits estimation of the k_e values from the structure of the compound which has been shown to correlate with other electrophilicity parameters often used, e.g., electron affinity and LUMO energy. The predictive performance of such electrophilic parameters is likely to vary because some reactivities probably result from induced properties (such as preferred polarization) on binding to biomolecules, while other chemicals may function more as co-carcinogens or promoters by modulating the reactivities of other chemicals, both endogenous and exogenous.

Interestingly, this method did surprisingly well in a predictive study (*20*), in spite of the fact that most known rodent carcinogens are believed to be activated by either cytochromes P4501 (*CYP1*) or P4502E1 (*CYP2E1*). A closer look at these two activating systems has, however, revealed that electrophilic properties of the substrates play an important role. The unique pivotal role of *CYP1* is due to its coordination with the regulatory Ah (dioxin) receptor previously mentioned, resulting in tissue concentrations of CYP1 being greatly augmented by enzyme induction. In particular, the dioxin-responsive *CYP1A1* gene is an interesting model system for analyzing the mechanism by which a protein complex such as the liganded receptor heteromer can trigger the chromatin structural changes that increase DNA accessibility (*38*). A key molecular triggering event in enzyme induction is chemical binding to the Ah receptor, and there is evidence that the planar aromatic substrates act as electron acceptors (electrophilic species) in charge transfer-type complexes with the Ah receptor (*39*). Bakale and McCreary (*40*) have emphasized the importance of electron-transfer processes as triggering events in metabolism of procarcinogens. Electrophilic compounds are more prone to reductive metabolism than nonelectrophilic ones. More recently, a study of the structural properties of some *CYP2E1* substrates has suggested an important role for reduction in the metabolism of these small molecules (*41*). Thus, there is evidence that electrophilic reactivity is important in key molecular triggering events associated with carcinogenic action of chemicals in rodents.

More research is also needed to develop *in situ* analytical (*42*) and computational chemistry (*43*) approaches aimed at quantitating and estimating relevant chemical reactivities within and across chemical classes associated with exposures, as well as for determining the concentrations of specific chemicals present. For example, the carbon column approach was described earlier as an approach to quantifying stacking reactivity for various aromatic chemicals. Finally, one must be aware that there may be undiscovered chemical reactivities that will prove to be toxicologically significant. New ways to measure and estimate chemical reactivites as a whole are also needed.

In conclusion, a hazard identification process that would permit assessment of reaction equivalents delivered to biological systems and target tissues and cells holds promise for grouping chemicals by common triggering mechanisms with clearly delineated linkages to toxic endpoints. An important objective is to more directly link chemical and molecular toxicology with the more established and extensively developed physical and chemical sciences, especially with regard to providing mechanistic insights. Such a process is not single-chemical limited and can permit easier detection of potential hazards by amplifying a common mechanistic signal associated with a mixture of chemicals. Structure groupings and activity classification have been discussed by others as an approach to the mixtures problem (*44*).

Mechanistic information naturally follows such an approach, permitting one to more readily develop PB-PK and BB-DR models for extrapolation purposes. In addi-

tion, such an approach can help in focusing attention and available resources on the most important exposure problem areas (reactivity hot spots). Electrophilic reactivity is further supported as an important factor in assessing how chemical structure determines toxicity. However, using toxicologically relevant molecular triggering events as a screen will ultimately require working backwards to identify specific chemicals to which we are exposed that need to be regulated. Similar to the toxic equivalency factor approach used for dioxinlike compounds, chemicals identified in a reactivity family could be quantitatively related through reactivity considerations to a prototypical compound (structure) for which the molecular triggering events (reactivity types estimated both experimentally or theoretically) for the toxicity of interest have been clearly delineated. This approach should not supplant other strategies incorporated into the risk assessment process. Nevertheless, in an era of cost consciousness and animal rights concerns, it may provide an alternative approach based on chemical structure to assess environmental exposures to chemicals and derive toxic equivalency factors.

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